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Effects of intense agricultural practices on heterotrophic processes in streams

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This study highlights the consequences of intensive agricultural practices on heterotrophic processes in streams along a strong gradient of perturbation.

Keywords:
Land use
Nutrient
Leaf litter breakdown
Macroinvertebrates
Hydrolasic activity

A B S T R A C T

In developed countries, changes in agriculture practices have greatly accelerated the degradation of the landscape and the functioning of adjacent aquatic ecosystems. Such alteration can in turn impair the services provided by aquatic ecosystems, namely the decomposition of organic matter, a key process in most small streams. To study this alteration, we recorded three measures of heterotrophic activity corresponding to microbial hydrolasic activity (FDA hydrolysis) and leaf litter breakdown rates with (k_c) and without invertebrates (k_f) along a gradient of contrasted agricultural pressures. Hydrolasic activity and k_f reflect local/microhabitat conditions (i.e. nutrient concentrations and organic matter content of the sediment) but not land use while k_c reflects land-use conditions. k_c, which is positively correlated with the biomass of Gammaridae, significantly decreased with increasing agricultural pressure, contrary to the taxonomic richness and biomass of Trichoptera and Plecoptera. Gammaridae may thus be considered a key species for organic matter recycling in agriculture-impacted streams.

1. Introduction

In most developed countries, changes in agriculture practices (type of cultivated crops, increase in nutrient loads) have greatly accelerated the alteration of the landscape (removing hedgerow, increasing field size) in the last 20–30 years. The contribution of agriculture to nutrient enrichment in rivers has been widely studied (Meybeck, 1982; Vitousek et al., 1997). Increases in nutrient contents, mostly nitrogen (Arheimer and Liden, 2000; Castillo et al., 2000; Goolsby et al., 2000; Huryn et al., 2002), phosphorus (Fisher et al., 2000; Bramley and Roth, 2002), and dissolved organic carbon (Ometo et al., 2000; Findlay et al., 2001) are among the most commonly reported agriculture impacts on streams.

The main drawbacks with the use of physico-chemical parameters for assessing agricultural impacts is their high temporal variability linked to both the hydrological cycle and agricultural practices (Randall and Mulla, 2001). In contrast, ecologists have long recognized that aquatic flora and fauna have the capacity to reveal a source of perturbation even if this source does not discharge pollutants, a feat impossible by chemical analysis alone (e.g. Hynes, 1960; Ometo et al., 2000; Townsend et al., 2004). For example, several studies have pointed out changes in assemblage structure and composition for both invertebrates and fishes following landscape modifications (see a review in Harding et al., 1998; Allan, 2004; Dolédec et al., 2006; Hagen et al., 2006), but changes in their role in ecosystem processes are rarely considered. Different microbial activities (Ainsworth and Goulder, 2000; Amann, 2000; Eisman and Montuelle, 1999; Montuelle and Volat, 1998) and biofilm primary production (e.g. for agriculture Corkum, 1996; Young and Huryn, 1999) have been shown to be efficient descriptors of river health and function. In recent years, there has been a growing interest in the use of leaf litter breakdown to assess the functional integrity of stream ecosystems (Gessner and Chauvet, 2002; Lecerf et al., 2006), but the effect of agriculture on heterotrophic processes has been evaluated only within a low range of perturbation (Young and Huryn, 1999; Hagen et al., 2006).

The originality of our study lies in the wide range of agriculture impacts considered and the combination of different methods to evaluate heterotrophic activities. Twelve sites were selected in three rivers with varying land uses, from forested areas (considered as a reference) to extensively and intensively cultivated areas. Three

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different measures of heterotrophic processes were used: sediment-associated hydrolasic activity, particulate organic matter breakdown resulting from either microbial activities or from both microbe and invertebrate activities. We hypothesized that agriculture (1) should increase microbial heterotrophic activities through an increase in nutrient availability for both sediment microbial activities (Claret et al., 2001) and litter breakdown (Suberkropp and Chauvet, 1995), but (2) should decrease shredder contribution to litter breakdown due to deleterious effects on invertebrates (Huryn et al., 2002; Lecerf et al., 2006). The invertebrate density, biomass and type of functional feeding group were considered in both the litter bags and the natural benthic litter. We hypothesized that rates of litter breakdown were linked to (3) the diversity of the invertebrate assemblages (Huryn et al., 2002) and (4) to the abundance and biomass of shredders (Benfield and Webster, 1985; Lecerf et al., 2006).

2. Material and methods

2.1. Study sites

The study area was located within the Long Term Socio-Ecological Research site of Pleine-Fougères (Brittany, Western France, Fig. 1). It consisted of a patchy landscape with forests, pastures, crop cultures and farming zones, exhibiting a wide gradient of agricultural pressure (Burel et al., 2003). Twelve sites were selected in four streams with similar riparian vegetation (with trees on one bank and grassland on the other, Table 1) and instream characteristics (e.g. local water velocity, discharge and geomorphology, Tables 1 and 2), but differing agricultural intensity in the catchment. Four sites were located in a stream receiving waste waters from a village (580 inhabitants) and industrial farming (chickens and cows). The upstream site was located above the waste water outflow in a forest (Villecartier) and was used as a reference site ("Petit Hermitage" stream: H1 in Fig. 1). The three other sites were located downstream the waste water outflow (H2, H3, H4 in Fig. 1). Four sites were located in a stream subjected to increasing agricultural pressure from up- to downstream ("Chênelais" stream: CH1, CH2, CH3, CH4 in Fig. 1) and three sites were located in a stream only affected by a gradient of increasing agricultural pressure [along up- to downstream or] ("Aleçon" stream: AL1, AL2, AL3 in Fig. 1). Finally, one site was located downstream of the waste water outflow of a town (1840 inhabitants) in an area dominated by crop fields and industrial cow and pig farming ("Jumelière" stream: JUM in Fig. 1). In the study region, the level of physico-chemical of streams is not directly related to their overall catchment land use (Sarriquet et al., 2006) because of the nutrient retention capacity of riparian wetlands and hedgerows (Montreuil and Mérot, 2006). We thus determined the stream water chemistry during a four-month period encompassing the leaf decomposition experiment and invertebrate sampling period.

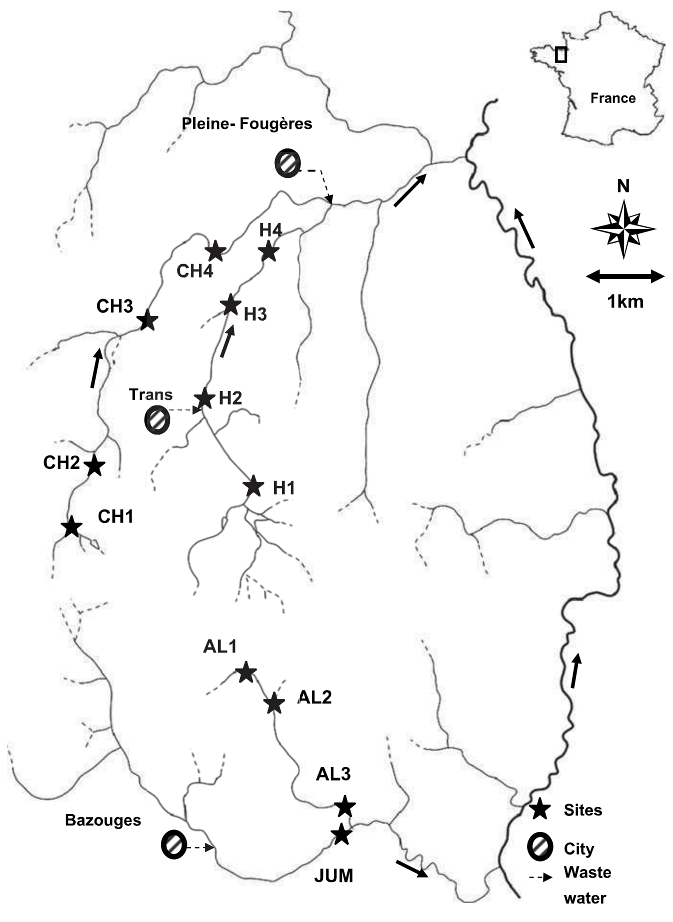

Fig. 1. Study area with the location of villages (hatched), waste water outflows (dotted arrows) and the 12 sampling sites (stars) located in four streams ("Petit Hermitage" stream: H1, H2, H3, H4; "Chênelais" stream: CH1, CH2, CH3, CH4; "Aleçon" stream: AL1, AL2, AL3; "Jumelière" stream: JUM).

Three water samples were collected at each site every two weeks from February to May 2005 and analyzed the same day. Water temperature, conductivity (LF92, WTW™, Weilheim, Germany), pH, and dissolved oxygen content (HQ20, HACH™, Dusseldorf, Germany) were measured in the field. In the laboratory, filtered-water samples (GF/C, 1.2 μm pore size, Whatman™, Maidstone, UK) were analyzed by colorimetric methods: molybdate-antimony for soluble reactive phosphorus (P-SRP; Murphy and Riley, 1962), indophenol blue for ammonium (N-NH_4^+; Rossum and Villaruz, 1963), diazotation for nitrite (N-NO_2^-; Barnes and Kollard, 1951) and after a reduction to nitrite by activated cadmium followed by nitrite titration for nitrate (N-NO_3^{-}; APHA, 1976).

2.2. Microbial activity in the sediment

Microbial activity in the sediment was estimated using the fluorescein diacetate (FDA) hydrolysis method (Schnurer and Rosswall, 1982; Fontvieille et al., 1992), where 0.1 ml of FDA and 3 ml of phosphate buffer (pH = 7.6) were added to 1–2 g of wet fine sediment (grain size < 200 μm) and incubated for 30–45 min at field temperature (Battin, 1997). Biological activity was stopped using 3 ml of pure acetone on ice. Supernatants were filtered with 0.45 μm cellulose membrane, rapidly frosted at −20 °C and kept frozen until analysis. Fluorescein concentration was calculated from the optical density of the filtered supernatant measured at 490 nm. The sediments used were dried during 24 h at 105 °C and total organic matter (TOM) concentration was measured by loss on ignition of dried sediment (4 h at 550 °C; Bretschhko and Leichtfried, 1988). Results were finally expressed as μmole of hydrolysed FDA per gram of dry sediment per hour or per gram of TOM per hour, the highest value corresponding to the highest microbial activity.

2.3. Leaf litter decomposition and invertebrate sampling

The litter-bag method (Chauvet, 1987; Boulton and Boon, 1991) was used to assess breakdown rate along the gradient of agricultural impact. Freshly fallen leaves of *Fagus sylvatica*, the dominant tree species in Northern Brittany woodlands and riparian forests, were collected in December 2004 from forests adjacent to the study sites. About 4 (±0.05) g of air-dried leaves were enclosed in coarse (6 mm mesh, 15 × 15 cm plastic bags) and 2 (±0.05) g in fine (0.5 mm mesh, 15 × 8 cm nylon bags) mesh bags closed in a quadratic shape. The coarse mesh allowed large shredders (such as Gammaridae and Limnephilidae) to enter the bag and feed on leaves, whereas fine mesh excluded most of the invertebrates without interfering with microbial colonization (Boulton and Boon, 1991). Fifteen bags per site were firmly tethered on the 17th of February 2005 to steel pegs placed within shallow riffles with similar current velocities (Table 1). Three bags of each type were collected at each site after 10, 24, 38, 52, and 66 days of immersion. To reduce invertebrate loss, a Surber net (0.5 mm mesh size) was placed downstream during leaf bag removal to collect dislodged animals only. Upon retrieval, the leaves were washed individually to remove sand, exogenous organic matter and invertebrates. The remaining leaf material from both types of bags was dried at 105 °C for 24 h and weighed to the nearest mg. Three control litter bags were used to estimate the initial dry mass of leaf litter after a short immersion time and drying at 105 °C for 24 h. Exponential litter breakdown rate (k) was calculated using the relationship,

$$W_t = W_i e^{-kt}$$

where W_t is the leaf dry mass remaining at the time t and W_i the leaf dry mass at the initial time (Petersen and Cummins, 1974). Calculations were performed separately for fine (k_f) and coarse mesh (k_c) bags. Invertebrates were preserved with 5% formaldehyde until identification. Invertebrates from coarse mesh bags were identified to species or genus except Nematoda, Oligochaeta, Hydracarina (not identified further), and most Diptera (to the family), counted, and preserved in 70% ethanol. The biomass of dominant group of shredders (i.e. Gammaridae, Trichoptera and Plecoptera) was determined separately from the other invertebrates by weighing dried (105 °C, 24 h) animals to the nearest 0.1 mg.

To compare invertebrate assemblages in litter bags and in the streams, four invertebrate samples were collected at each site in benthic litter on the 4th and 5th April 2005. The litter was removed to a depth of about 5 cm using a Surber net

Table 1
Environmental characteristics of the 12 sites located on the "Petit Hermitage" (H1–H4), "Chênelais" stream (CH1–CH4), and "Aleçon–Jumelière" (AL1–JUM) streams. Riparian vegetation directly surrounding the stations on right and left banks is noted as forest (F), hedgerow (H), or grassland (G). Distance to waste water outflows from villages or industrial farming is indicated when applicable.

Site	Stream order	Water velocity ($m\,s^{-1}$)	Riparian vegetation	Distance to waste water outflows (km)
H1	2	0.25 ± 0.09	F + G	–
H2	2	0.33 ± 0.04	H + G	0.3
H3	2	0.42 ± 0.07	G + H	1.4
H4	2	0.40 ± 0.07	G + H	2.7
CH1	2	0.23 ± 0.04	H + G	–
CH2	2	0.18 ± 0.08	H + G	–
CH3	3	0.23 ± 0.07	G + H	–
CH4	3	0.43 ± 0.11	G + H	–
AL1	1	0.26 ± 0.11	G + H	–
AL2	1	0.23 ± 0.11	G + H	–
AL3	2	0.46 ± 0.16	H + G	–
JUM	3	0.24 ± 0.14	G + H	2.4

(0.05 m^2, 0.5 mm mesh size). Samples were fixed with 5% formaldehyde in the field. In the laboratory, organisms were sorted, identified at the same taxonomic level as invertebrates collected in the leaf litter bags, counted, and preserved in 70% ethanol.

To characterize invertebrate assemblages in leaf litter bags and to compare them with instream assemblages (Surber samples), taxonomic richness, abundance, and Simpson dominance index (see Magurran, 1988 for details) were calculated for each sample considering the total fauna list on one hand and shredders only (according to Tachet et al., 2000) on another hand.

2.4. Statistical analysis

We performed a PCA on the average nutrient contents (nitrates, nitrites, ammonium and soluble reactive phosphorus). Since the first two PCA axes delivered most of the variability (i.e. 74.6%), we used these axes to classify sites by cluster analysis (Euclidean distance and Ward's aggregation method) on the first two axes of a PCA on the average nutrient contents (nitrates, nitrites, ammonium and soluble reactive phosphorus). This enabled a global grouping of sites along the gradient of agricultural practices and resulted in a land-use typology. The significance of the overall difference (between-group variances) was tested against simulated values obtained after 1000 permutations of the rows of the water chemistry table (Romesburg, 1985). Analyses were performed using ade4 library implemented in R freeware (e.g. Ihaka and Gentleman, 1996).

Leaf breakdown rates were determined by non-linear regression (Petersen and Cummins, 1974). We used the Spearman's Rank correlation to highlight the relationships between land-use types and heterotrophic processes (hydrolasic activity, water chemistry, leaf litter breakdown rates k_c and k_f) and faunal assemblages (biocenotic indices and biomass of invertebrates) in leaf litter bags and in Surber net samples.

Kruskal–Wallis tests were performed to highlight differences among land-use types for hydrolasic activity and invertebrate abundance in litter bags. Changes between two pair-wise comparisons were investigated through a *post-hoc* analysis based on multiple comparison tests (Siegel and Castellan, 1988) using procedures from Statistica 7.1 (StatSoft, 2001).

3. Results

3.1. Water chemistry and microbial activity in sediment

According to cluster analysis based on the average nutrient contents, sites were classified into four land-use groupings. A first grouping (Fig. 2) included the four sites downstream of waste water outflows (JUM, H2, H3, H4) which had high N-NH_4^+, N-NO_2^-, and P-SRP concentrations (Table 2). The second group incorporated sites not directly influenced by waste waters and could be divided into three other groups according to the intensity of agricultural activities in the watershed (Fig. 2): (i) the forested site with low nutrient concentrations (H1), (ii) the four sites located in traditional hedged farming area (AL1, AL2, CH1, CH2) with intermediate nutrient concentrations, and (iii) the three sites located in the more intensively exploited area (CH3, CH4, AL3) with high nitrate and intermediate N-NH_4^+, N-NO_2^-, and P-SRP concentrations (Table 2).

Between-class PCA performed on the nutrient parameters (N-NH_4^+, N-NO_2^-, N-$NO_3^-$and P-SRP) showed that site groupings (above) explained significantly more variability (observed variability between site groupings = 34.6%, simulated p-value < 0.001) than stream groupings (observed variability between streams = 11.6%, simulated p-value < 0.001) and was further used as a proxy for land uses. Our final land-use typology thus resulted into four groupings that combine all anthropogenic disturbances at the catchment scale (gradients in agriculture activities and local enrichment in nutrients): forested area (noted F in Table 2), traditional hedged farming (HF, Table 2), intensive agriculture (IA, Table 2), and intensive agriculture with waste water outflows (IA + WW, Table 2).

3.2. Hydrolasic activity

FDA hydrolysis microbial activity ranged from 0.004 to 0.323 μM/g/h when reported as sediment dry mass. It was significantly and positively correlated with the total organic matter content of the sediment ($r = 0.776$; $p < 0.003$, Fig. 3a). To take into account the local effect of organic matter content, hydrolasic microbial activity was also reported as organic matter dry mass. It ranged from 0.33 to 6.58 μM/gOM/h, but did neither vary

Table 2
Water chemical characteristics (mean ± SD) of the 12 studied sites and groups of sites taken from a cluster analysis (last line in bold), with F = Forest; HF = Hedged Farmland; IA = Intensive Agriculture; IA + WW = Intensive Agriculture + Waste Water.

	Temperature (°C)	Dissolved O_2 ($mg\,l^{-1}$)	pH	Conductivity ($\mu S\,cm^{-1}$)	N-NO_3^- ($\mu gN\,l^{-1}$)	N-NO_2^- ($\mu gN\,l^{-1}$)	N-NH_4^+ ($\mu gN\,l^{-1}$)	P-SRP ($\mu gP\,l^{-1}$)
F (H1)	**8.7** ± **3.4**	**10.9** ± **1.2**	**7.0** ± **0.4**	**159** ± **5**	**510** ± **19**	**10** ± **8**	**49** ± **18**	**19** ± **8**
CH1	8.2 ± 3.3	10.5 ± 1.5	6.9 ± 0.2	326 ± 16	2820 ± 173	23 ± 6	80 ± 43	29 ± 15
CH2	8.1 ± 3.1	10.7 ± 1.4	6.9 ± 0.3	331 ± 17	3070 ± 164	24 ± 7	87 ± 50	36 ± 20
AL1	8.2 ± 2.9	11.3 ± 1.3	7.1 ± 0.3	261 ± 7	2720 ± 127	17 ± 6	86 ± 66	40 ± 16
AL2	8.2 ± 3.1	10.6 ± 1.5	6.9 ± 0.4	271 ± 13	3710 ± 193	18 ± 9	91 ± 50	30 ± 11
HF	**8.2** ± **3.0**	**10.8** ± **1.4**	**6.9** ± **0.3**	**297** ± **34**	**3080** ± **167**	**20** ± **8**	**86** ± **52**	**34** ± **16**
CH3	8.5 ± 3.3	11.4 ± 1.4	7.4 ± 0.3	349 ± 16	4080 ± 195	18 ± 6	50 ± 20	33 ± 10
CH4	9.1 ± 2.9	11.1 ± 1.2	7.4 ± 0.4	391 ± 14	4920 ± 157	22 ± 5	62 ± 11	28 ± 6
AL3	8.4 ± 2.8	11.8 ± 1.6	7.0 ± 0.4	303 ± 13	4900 ± 189	12 ± 6	65 ± 38	32 ± 11
IA	**8.5** ± **3.0**	**11.5** ± **1.4**	**7.3** ± **0.4**	**349** ± **40**	**4630** ± **182**	**17** ± **7**	**59** ± **26**	**31** ± **9**
H2	8.8 ± 3.2	10.7 ± 1.3	7.0 ± 0.3	298 ± 34	2770 ± 117	39 ± 7	320 ± 136	57 ± 23
H3	8.5 ± 3.3	11.7 ± 1.3	7.3 ± 0.2	300 ± 33	3350 ± 184	38 ± 8	146 ± 92	48 ± 19
H4	8.6 ± 3.2	11.0 ± 1.3	7.4 ± 0.2	321 ± 39	3300 ± 144	33 ± 8	116 ± 54	46 ± 13
JUM	8.7 ± 3.0	11.8 ± 1.8	7.1 ± 0.4	344 ± 21	6490 ± 225	42 ± 14	97 ± 83	95 ± 55
IA + WW	**8.7** ± **3.1**	**11.1** ± **1.5**	**7.2** ± **0.3**	**316** ± **36**	**3980** ± **224**	**38** ± **10**	**170** ± **129**	**61** ± **37**

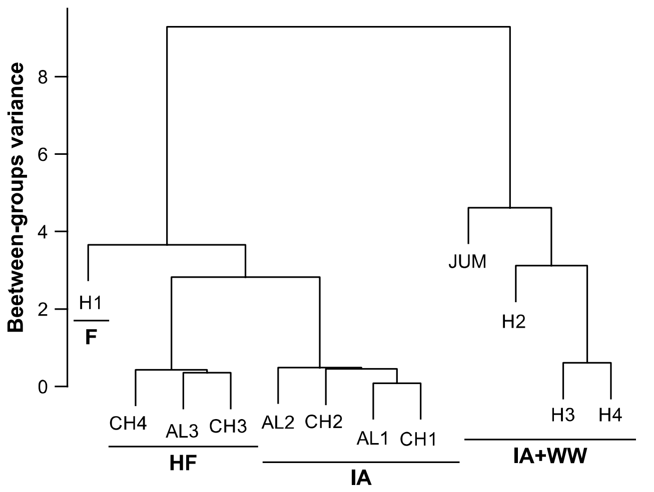

Fig. 2. Result of a cluster analysis performed on the first two axes of a PCA of the average nutrient content of each site using Ward's agglomerative method and the resulting classification by land-use type (F = Forest; HF = Hedged farmland; IA = Intensive agriculture; IA + WW = Intensive agriculture plus waste water).

significantly along the upstream–downstream gradient (data not shown), nor according to the types of stations ($r = 0.194$; $p = 0.546$; Fig. 3b) and nitrate concentrations ($r = -0.364$; $p = 0.245$). However, hydrolasic microbial activity was significantly and positively correlated with N-NH_4^+ ($r = 0.594$; $p = 0.041$), N-NO_2^- ($r = 0.594$; $p = 0.041$), and P-SRP concentrations ($r = 0.622$; $p = 0.031$).

3.3. Litter breakdown due to microbial activity

Leaf litter breakdown rate in fine mesh bags (k_f) was negatively correlated with N-NH_4^+ ($r = -0.801$; $p = 0.002$), N-NO_2^- ($r = -0.648$; $p = 0.023$) and P-SRP ($r = -0.676$; $p = 0.016$) concentrations, but not with nitrate concentrations ($r = 0.014$; $p = 0.965$). This breakdown rate did not significantly change among land-use types (Fig. 4a; $r = 0.526$; $p = 0.079$).

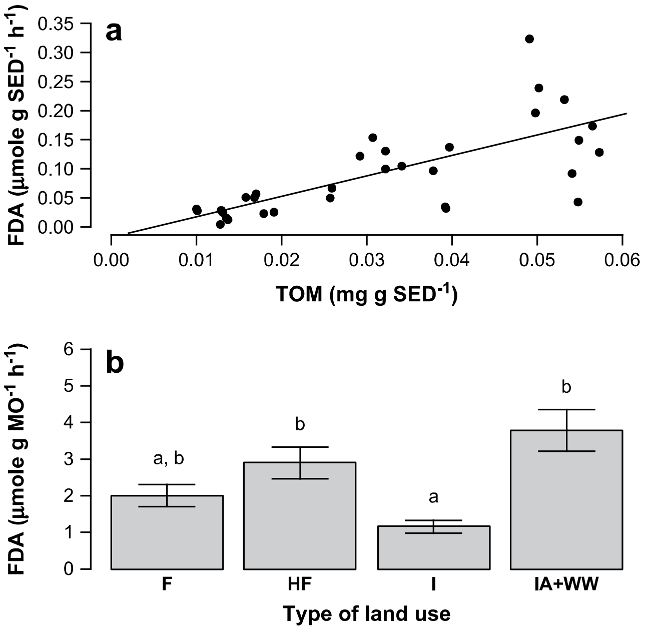

Fig. 3. (a) Relationship between hydrolasic microbial activity (FDA hydrolysis reported per gram of dry sediment per hour) and the total organic matter content of the sediment (mg TOM per gram of dry sediment) ($R^2 = 0.51$, $y = 3.34x - 0.01$, $p < 3.10^{-6}$). (b) Changes in hydrolasic microbial activity (mean ± 95% SE, expressed in FDA hydrolysis per gram of TOM per hour) according to the four types of land use (F = Forest; HF = Hedged farmland; IA = Intensive agriculture; IA + WW = Intensive agriculture plus waste water). For each grouping, significant between-type differences (multiple comparison tests following the Kruskal–Wallis test) in hydrolasic microbial activities are indicated by different letters.

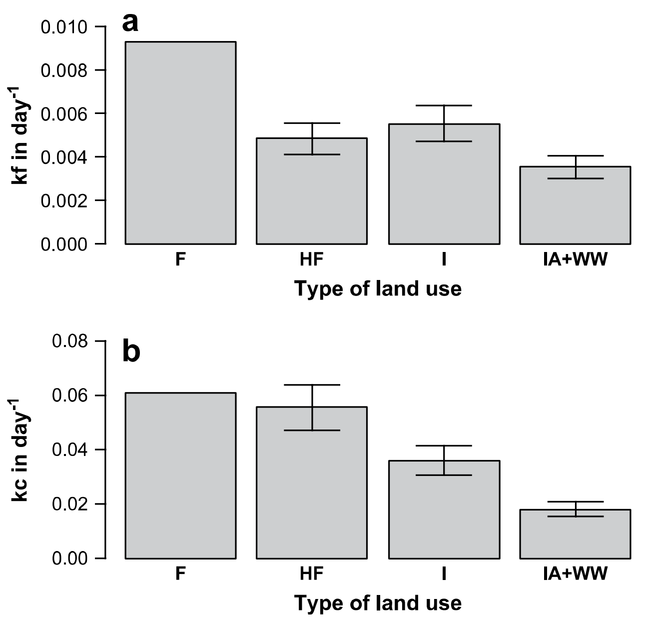

Fig. 4. Mean values (±SE) of leaf litter breakdown rates in fine (k_f) and coarse (k_c) mesh bags in the four land-use groupings (F = Forest; HF = Hedged farmland; IA = Intensive agriculture; IA + WW = Intensive agriculture plus waste water).

3.4. Litter breakdown and invertebrates

No correlation was found between the leaf litter breakdown rate in coarse mesh bags (k_c) and nutrient concentrations ($r < 0.552$; $p > 0.0625$). However, we observed a significant decrease of k_c among sites based on anthropogenic disturbances (Fig. 4; $r = 749$; $p = 0.005$): k_c decreased by 75% between the reference forested site (i.e. H1) and the most disturbed sites (i.e. JUM).

We did not find any correlations between k_c and the mean richness ($r = 0.119$; $p = 0.713$), abundance ($r = 0.007$; $p = 0.983$), or with the Simpson dominance index ($r = 0.238$; $p = 0.457$) of invertebrates in the litter bags. No significant correlations were found between k_c and the mean taxonomic richness of shredders,

Table 3
Taxonomic richness and abundance of invertebrates in coarse mesh litter bags according to the four site groupings (F = Forest; HF = Hedged Farmland; IA = Intensive Agriculture; IA + WW = Intensive Agriculture + Waste Water). *P*-values refer to Spearman rank correlations between leaf litter breakdown rates in coarse mesh bags (k_c) and invertebrate metrics.

	F, $n = 9$	HF, $n = 27$	IA, $n = 27$	IA + WW, $n = 36$	*P*-values
Total richness	6.3 ± 2.6	13.6 ± 4.1	17.3 ± 5.2	13.8 ± 4.9	NS
Total abundance	67 ± 29	157 ± 164	104 ± 49	141 ± 77	NS
Shredder richness	3.0 ± 1.2	4.9 ± 2.1	6.4 ± 1.8	4.4 ± 2.5	NS
Shredder abundance	62 ± 28	53 ± 28	45 ± 24	24 ± 23	<0.001
Scraper richness	1.7 ± 1.4	3.6 ± 2.2	4.1 ± 2.1	2.9 ± 1.3	NS
Scraper abundance	2.2 ± 1.9	37 ± 31	44 ± 40	83 ± 60	NS
Deposit-feeder richness	0.9 ± 0.6	1.2 ± 1.1	1.9 ± 1.4	1.9 ± 0.7	NS
Deposit-feeder abundance	1.4 ± 1.3	2.8 ± 3.5	4.1 ± 4.0	22 ± 19	NS

and the mean taxonomic richness and abundance of deposit feeders and scrapers (Table 3; $r < 0.454$; $p > 0.138$). However, k_c was significantly and positively correlated with the mean abundance of shredders in litter bags ($r = 0.84$; $p < 0.001$), which significantly decreased from the forested site to the most disturbed type of stations with 62 ± 28 and 24 ± 23 individuals per bag (Table 3; Kruskal–Wallis test; $N = 108$; $p < 0.001$).

The biomass of Gammaridae (here a mix of *Gammarus pulex* and *Echinogammarus berilloni*) sampled in the litter bags was highly and positively correlated with breakdown rates in coarse mesh bags (Fig. 5a; $r = 0.741$; $p < 0.006$), whereas no relationships were observed with Trichoptera, Plecoptera or total invertebrate biomasses ($r < 0.490$; $p > 0.11$). The abundances of Gammaridae in litter bags also decreased from the reference site to the most disturbed sites with 56.2 ± 15.2 and 5.8 ± 2 individuals per bags, (Fig. 5b; Kruskal–Wallis test; $N = 108$; $p < 0.001$). Similarly, their relative biomass decreased from $85.4 \pm 9.4\%$ in the forest site to $15.1 \pm 17.9\%$ in the most disturbed sites (Kruskal–Wallis test; $N = 108$; $p < 0.001$).

The richness and abundance of invertebrates in litter bags and in benthic litter accumulations (i.e. Surber samples) were not correlated with land-use type ($r < 0.402$; $p > 0.195$). Richness and Simpson's dominance of invertebrates in litter bags were positively correlated with the richness and dominance in benthic litter accumulations (respectively, $r = 0.676$; $p = 0.016$ and $r = 0.654$; $p = 0.029$). However, invertebrate abundance in litter bags and in benthic litter was not correlated ($r = 0.294$; $p = 0.354$). Gammaridae abundance sampled in the litter bags and benthic litter was highly and positively correlated ($r = 0.874$; $p < 0.001$). Furthermore, Gammaridae abundance was negatively correlated with the type of land use ($r = -0.734$; $p = 0.006$ for the abundance in litter bags and $r = -0.752$; $p = 0.005$ in benthic litter). Among shredders, Gammaridae abundance decreased significantly from the reference site to the most disturbed stations, whereas abundance of most Trichoptera [mainly *Mystacides azurea* (Leptoceridae) and *Lasiocephala basalis* (Lepidostomatidae)] increased (Table 4).

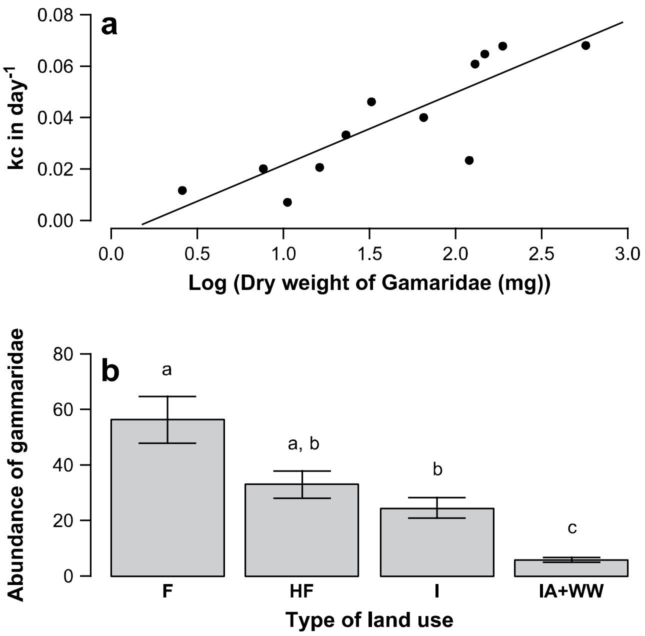

Fig. 5. (a) Relationship between the mean biomass of Gammaridae (log 10-transformed) in each site and the leaf litter breakdown rates in coarse mesh bags (k_c) ($R^2 = 0.72$, $y = 0.028x - 0.007$, $p < 5 \times 10^{-4}$). (b) Average abundance (mean ± SE) of Gammaridae for the different types of land use (F = Forest; HF = Hedged farmland; IA = Intensive agriculture; IA + WW = Intensive agriculture plus waste water). For each type, significant between-type differences (multiple comparison tests following the Kruskal–Wallis) in breakdown rates are indicated by different letters.

4. Discussion

In this study, we observed a strong upstream–downstream gradient in nutrient concentration of stream water, partly due to agricultural intensification (for nitrate, already highlighted by several works: e.g. Watzin and McIntosh, 1999; Bramley and Roth, 2002; Gergel et al., 2002; Huryn et al., 2002; Roy et al., 2003), but also linked to waste water outflows of villages and industrial farming (for N-NH_4^+, N-NO_2^-, and P-SRP; already documented by Xia et al., 2002; see also Brainwood et al., 2004, for farmland waste waters). Released N-NH_4^+ and N-NO_2^- probably decreased through nitrification, which contributed in part to increased nitrate concentrations downstream. In addition to this general trend, we observed strong temporal variability in nutrient concentrations, emphasizing that chemical characteristics are variable and difficult to rely on for assessing the quality of agricultural streams. In such cases, biological processes may be of greater use than water chemical characteristics *per se*.

4.1. Microbial processes

The hydrolasic activity of bottom sediment as determined by FDA hydrolysis did not change between the four land-use types. This activity was significantly correlated with the total organic matter content of the sediments and point-source pollutions as a result of the villages and industrial farming (here the N-NH_4^+, N-NO_2^-, and P-SRP concentrations). The latter observation supported our first prediction concerning the increase of microbial heterotrophic activities through an increase in nutrient availability. The enhancement of microbial activities by increased nutrient availability is well documented (e.g. Brookes et al., 2000; Mutuku-Mathooko et al., 2002). Furthermore, hydrolasic activity is known to be strongly linked to the available organic matter in bottom sediments (Marmonier et al., 1995; Claret et al., 1998) and to the exchanges between surface and interstitial waters (Claret et al., 2001). In this study, the hydrolasic activity is thus more closely related to characteristics at a local scale than to large-scale agricultural patterns.

When large invertebrates were not present from litter bags (i.e. in fine mesh bags), we did not observe significant changes in litter breakdown rates due to microbial and meiofauna activities (k_f) with the types of stations, but strong negative relationships between k_f and N-NH_4^+, N-NO_2^-, and P-SRP concentrations (i.e. in relation with the local context of villages and industrial farming). This result is not consistent with our first hypothesis and other studies that highlighted significant increases in microbial biomass and activity together with increasing nutrient contents in stream located in poorly disturbed or pristine rivers (Suberkropp and Chauvet, 1995; Tank and Webster, 1998; Tank and Dodds, 2003) or impacted by waste water inputs where microbial decomposers were poorly affected (Lecerf et al., 2006). In our case, the extremely high values of N-NO_2^- and N-NH_4^+ measured in the most disturbed stations (mean values of 0.035 mg N l^{-1} and 0.109 mg N l^{-1} respectively, see Table 2) may have reached inhibitive thresholds for this heterotrophic process (e.g. Baldy et al., 2007).

In the same way, we did not observe any significant relationships between nitrate concentrations and k_f, while Suberkropp and Chauvet (1995) and Huryn et al. (2002) highlighted a positive correlation. This apparent contradiction may be linked to the strong degradation of surface and ground waters in Brittany (French Water Agency, http://www.eau-loire-bretagne.fr/) and the very high nitrate concentrations in our most disturbed stations (up to 19.0 mg N l^{-1}, Table 2), that are well above those reported from other studies (e.g. concentrations always lower than 0.8 mg N l^{-1} in Huryn et al., 2002). In addition, high nitrate concentrations were associated with high N-NH_4^+ and N-NO_2^- concentrations. A positive

Table 4
Correlation between land-use types and abundances of the main taxonomic groups of shredders associated with benthic litter accumulations (F = Forest; HF = Hedged Farmland; IA = Intensive Agriculture; IA + WW = Intensive Agriculture + Waste Water). *P*-values refer to Spearman rank correlations between invertebrate metrics and the type of land use.

		F, $n = 4$	HF, $n = 12$	IA, $n = 12$	IA + WW, $n = 16$	Spearman *R*	*P*
Crustacea	Asellidae	2 ± 2	9.3 ± 9	2.9 ± 5	7.5 ± 11	–	NS
	Gammaridae	313 ± 160	92 ± 67	122 ± 89	23 ± 25	−0.66	<0.001
Diptera		0	0.2 ± 0	0.4 ± 1	0.3 ± 0	–	NS
Plecoptera		0	5.8 ± 8	0.4 ± 1	0.3 ± 1	–	NS
Trichoptera	Leptoceridae	0	0	3.3 ± 6	3.9 ± 8	0.32	0.029
	Limnephilidae	54 ± 13	32 ± 24	22 ± 25	32 ± 25	–	NS
	Others	11.5 ± 5	2.6 ± 3	13.3 ± 9	49 ± 71	0.53	<0.001

linear relationship between nitrate and k_f [as observed by Suberkropp and Chauvet, 1995] may have been masked by the inhibiting effect of N-NH_4^+, N-NO_2^- and/or xenobiotics (e.g. Fungicides) in our most perturbed sites. Such antagonistic effects as suspected in the present study may result in a non-linear response of k_f to a broad range of anthropogenic pressures, which complicate the use of microbial breakdown rate as an indicator of agricultural stream ecosystem impairment.

4.2. *Litter breakdown and invertebrates*

In contrast to processes linked to microbial and meiofaunal activities, we observed no relationships between litter breakdown rates in coarse mesh bags (k_c) and local chemical characteristics, but strong differences of k_c among land-use types (with a decrease by 75% between the reference and the most disturbed stations). This result supports our second prediction that agricultural practices would result in negative effects on invertebrates, and in particular on shredders. The decreasing rate of litter breakdown with increasing agricultural intensity was indeed significantly correlated with a decrease in shredder abundance (supporting our fourth prediction). Several studies have related breakdown rates to the shredder density or biomass (Benfield and Webster, 1985; Fabre and Chauvet, 1998; Niyogi et al., 2001; Huryn et al., 2002) and highlighted the role of shredders in controlling litter breakdown in different land-use contexts including agricultural areas (Lecerf et al., 2006; Hagen et al., 2006). For example, Sponseller and Benfield (2001) found that breakdown rates of sycamore leaves were correlated with shredder biomass related to streambed particle size and to the land use in the surrounding catchment.

In this study, the breakdown rate was correlated with the shredder biomass and abundance in accord with our fourth prediction, but not with shredder taxonomic richness (in contradiction with our third prediction and findings of Huryn et al., 2002). Among shredders, only the biomass of Gammaridae was significantly correlated with breakdown rates. These two results suggest that there is no functional redundancy in the litter consumption among shredder species in the studied streams and the decrease of Gammaridae along the gradient of land use was not compensated for by the activity of other taxonomic groups. Gammaridae hence appears as the key species for the leaf litter breakdown processes in these rural streams. However, the *per capita* efficiency of Gammaridae appears low relative to other shredders (Jonsson et al., 2002). This implies that freshwater amphipods control leaf litter breakdown, and indirectly the diversity–function relationship, primarily through their high abundance (they represented 85% of the invertebrate community at the reference site) rather than by high-energy conversion efficiency. This hypothesis is supported by the observed decrease in k_c and Gammaridae abundances with agricultural intensification in both litter bags (Fig. 4) and benthic litter accumulations (Table 4). Sensitivity of Gammaridae to nutrient concentrations was experimentally demonstrated for nitrate (Camargo et al., 2005), nitrite (Alonso and Camargo, 2006), and phosphorus (McCormick et al., 2004), all being strongly associated with anthropogenic disturbances (i.e. agricultural practices like industrial farming).

The abundance and diversity of invertebrates in litter bags were highly correlated with those in benthic litter accumulations. The impact of agricultural practices is thus similar for assemblages sampled in the litter bags and in the benthic habitat. However, we observed in benthic litter accumulations an increase of some Trichoptera (mainly *M. azurea* and *L. basalis)* not observed in litter bags. *M. azurea* fed on living macrophyte (Tachet et al., 2000), which tend to increase with increasing agriculture in Brittany (Haury and Aïdara, 1999). The increase in the abundance of *L. basalis* is difficult to explain because of the sensitivity of this species to high nutrient concentrations (Bonada et al., 2004). A possible explanation might be the observed decrease in Gammaridae, which may result in changes in the assemblage composition as already observed in other rural streams in Brittany (Sarriquet et al., 2007).

5. Conclusion

In conclusion, our study suggests that microbial processes are strongly influenced by microhabitat conditions, and thus are ineffective indicators of agricultural disturbances. In contrast, the total litter breakdown (as represented by k_c) may be an efficient tool to evaluate the effect of anthropogenic disturbances occurring at the catchment scale, e.g. linked to the agricultural intensification. Here, the values of k_c were strongly controlled by one group of shredders, the Gammaridae, which can be considered as the key organisms controlling litter breakdown. In addition, redundancy among shredder species was absent or very weak in the studied streams. The hypothesis of Huryn et al. (2002) that an assemblage of species sensitive to disturbance can be functionally replaced by an assemblage of organisms more tolerant to declining water quality is thus not supported by our results in the Brittany streams. The lack of functional redundancy (Covich et al., 1999; Mermillod-Blondin et al., 2001) in this agricultural area may be a major factor that induces dysfunctioning of heterotrophic processes in streams affected by intense agriculture. As a result, future work should focus on the efficiency of rare species in controlling litter breakdown in sites where Gammaridae are excluded for other reasons (e.g. in temporary streams).

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